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Cadherins are the primary regulators of the overall state of epithelial cell contact and facilitate an appropriate cytoskeletal organization and the establishment of many other kinds of cell interactions that preserve tissue integrity, thereby leading to the establishment of the density-dependent inhibition of growth. However, it remains obscure whether E-cadherin directly transfers growth inhibitory signals to the cells, or if other types of molecular cell interactions indirectly influenced by the establishment of cadherin mediated cell contacts, are responsible for contact inhibition of growth. In this study I selectively activate the formation of E-cadherin homophilic adhesive bonds, using a specific recombinant protein to engage E-cadherin molecules at the cell surface of dispersed primary breast epithelial cells. Here I show evidence that E-cadherin ligation is capable to reduce the rate of cells entered into S-phase, in a process not linked with apoptosis. β-catenin/TCF signaling activity does not appear to be involved in the inhibition of cell growth, since the cells did not displayed constitutive β-catenin/TCF signaling. Moreover, direct inhibition of β-catenin/TCF signaling pathway was not able to decrease the proliferation rate in these cells, suggesting the involvement of other growth inhibitory signaling pathways in this event.

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Table of Contents

Cover.....1

SF 298.....2

Introduction.....4

Body.....4

Key Research Accomplishments.....9

Reportable Outcomes.....9

Conclusions.....9

References.....10

Appendices.....13

Role of E-cadherin homophilic contacts in the inhibition of cell growth of primary breast cells.
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INTRODUCTION

Continued expression and functional activity of E-cadherin are required for cells to remain tightly associated in the epithelium and for the maintenance of the tissue integrity, thereby leading to the establishment of the density-dependent inhibition of growth in normal epithelial cells. The loss or significant reduction of E-cadherin expression has been strongly implicated in the pathogenesis of breast cancer, and other epithelial tumors.
Reconstitution of E-cadherin expression has been found to slow the growth of cancer cells. However, until now it has not been clear if E-cadherin by itself is able to transfer growth inhibitory signals to the cells or if the participation of other factors, that are indirectly influenced by the establishment of cadherin mediated cell contacts, are responsible for cell growth inhibition. In this work, I am studying the direct role of E-cadherin in the generation of cell growth inhibitory signals to clarify this important issue. This will open new avenues to understand the normal behavior of the cells, and the mechanisms that could provoke the aberrant signals that induce aggressive breast cancer. Normal epithelial cells are being used in this study, in isolated manner to ensure the adhesion contacts are only mediated by E-cadherin. The results obtained will be the basis to identify new elements that are associated with tumor aggressiveness that could be useful for the design of directed therapeutic approaches.

BODY:

Task 1. Analyze whether the engagement of E-cadherin in a homophilic adhesive bond is capable of transducing a growth inhibitory signal. (Months 1-6)

E-cadherin Ligation Is Capable to Reduce the S-phase Entry of Primary Epithelial Cells

To measure the direct effect of E-cadherin on cell proliferation, early passages of human mammary epithelial cells (HUMEC), were grown at subconfluence to avoid cell-cell interactions and under conditions in which the cells received strong growth stimulatory signals, from serum in the medium and from plating cells on fibronectin. Cells were harvested prior to plating under conditions that minimize removal of the surface E-cadherin. Cadherin molecules present at the cell surface were specifically engaged using microspheres or dishes coated with purified and functionally active chimeric Fc-hE-cadherin (Fc-hE), and antibodies against other cell surface proteins such as class I MHC (HLA, β2-microglobulin) and the Na^+K^+ ATPase β1 subunit were used as controls for ligand specificity attachment (Figure 1A and B). Similar approaches have been previously used to explore cadherin function and regulation (Zhong et al., 1999; Kovacs et al., 2002; Lambert et al., 2002). Proliferating cells were labeled with 5-bromodeoxyridine (BrdU) and the percentage of proliferating cells after 24 h and 48 h was determined measuring the incorporation of BrdU by immunofluorescence. When E-cadherin homophilic ligation was activated at the cell surface with either of two Fc-hE coated microspheres (Figure 1C) or coverslips (Figure 1D), specific and substantially reduced levels of BrdU incorporation were observed, as compared to ligation controls. The latter excludes the possibility that the observed growth inhibitory effect mediated by Fc-hE coated microspheres could have been due to their engulfment setting up cytoskeletal and morphological changes and not due to the specific and homophilic engagement of these molecules at the cell surface. To tritiate the efficiency of Fc-hE binding onto cell surface and the strength of the growth inhibitory signal triggered by E-cadherin ligation, I coated coverslips (data not shown) and microspheres with the medium (Figure 1E) and

minimum (Figure 1F) loading capacity of the beads obtaining equivalent results in the percentage of cells in S-phase to those with the maximum loading capacity of the beads (Figure 2C). I also observed a growth inhibitory effect when an antibody against the extracellular domain of E-cadherin was used (HECD-1, data not shown). Thus, this strongly suggests that rearrangement or clustering of E-cadherin molecules on the cell surface is necessary to transmit the growth inhibitory signal to the cells.

E-cadherin Ligation Inhibits Proliferation of Certain Cell Lines

In order to obtain further insights about the strength nature of the E-cadherin growth inhibitory signal I decided to explore whether the activation of the homophilic ligation of E-cadherin is also capable to reduce the proliferation of certain cell lines. I selected the human breast adenocarcinoma MCF-7 and the human colocarcinoma HT29 cell lines because they differentiate well in culture, express endogenously E-cadherin and can be maintained at low cell density in culture. I also used the transfectant cell line CHO-hE-cadherin generated previously in Dr. Gumbiner's laboratory (Gottardi et al., 2001). The engagement of E-cadherin into homophilic adhesive contacts at the cell surface using both Fc-hE coated microspheres or coverslips (data not shown) was able to reduce the percentage of HT29 (Figure 2A) and MCF-7 cells (Figure 2B) in S-phase, even when minimum and medium loading amounts of protein were used (data not shown). On the contrary, CHO-hE-cadherin cells did not reduce their growth rates after the engagement of E-cadherin (Figure 2C). This could be explained by the fact that this is a highly transformed cell line and the solely reintroduction of E-cadherin is not enough to activate the cellular machinery that work in an orchestrated manner to transmit the cell growth inhibitory signal mediated by E-cadherin ligation. These findings are not consistent with those observed when VE-cadherin was expressed in these cells, suggesting that different mechanisms may account for the growth inhibitory activity mediated by VE-cadherin (Caveda et al., 1996).

The E-cadherin growth inhibitory effect is not related with an increase of apoptosis

My experiments of activation of E-cadherin ligation suggested that this event directly modulate growth independently of other intercellular interactions. To investigate whether these results were not secondary to an increase in the cellular apoptosis rate, I examined this issue using the terminal deoxytransferase-mediated deoxyuridine nick end-labeling (TUNEL) assay. In all cases, cultures were treated with microspheres coated at their maximum loading capacity. As shown in Table I, the engagement of E-cadherin onto the cell surface did not alter the number of apoptotic cells, compared to ligation controls. These data show that, under the experimental conditions used in this study the percentage of TUNEL positive cells is minor and there were not considerable differences between the apoptosis rate of the cells coated with Fc-hE microspheres compared to controls.

Task 3. Identify whether the growth inhibitory signal mediated by E-cadherin homophilic contacts is mediated through Wnt/β-catenin signaling antagonism (Months 6-18)

Specific Engagement of E-cadherin Inhibits Cell Growth Even When Cells Do Not Display β-catenin/TCF Transcriptional Activity

In addition to their role in cell adhesion, β-catenin has a well-established role as an essential mediator of the canonical Wnt pathway (Polakis, 2001; Seidensticker and Behrens, 2000). When this pathway becomes activated, β-catenin is stabilized in the cytoplasm evading its interaction with components that regulate β-catenin turnover such as glycogen synthase kinase-3,

adenomatous polyposis coli (APC) or Axin, and its consequent degradation by proteasomes. As a result, β-catenin accumulates in the cytoplasm and is able to interact with lymphocyte enhancer factor/T cell factor (Lef/TCF) family of transcription factors facilitating its import into the nucleus modulating the expression of certain genes, some of them implicated in cell growth control, such as cyclin D1 and c-myc (He et al., 1998; Shtutman et al., 1999; Tetsu and McCormick, 1999).

This raises the ostensible possibility that the growth inhibitory activity directly triggered by E-cadherin ligation depends mainly on a β-catenin/TCF activity downregulation, acting by binding cytoplasmic β-catenin and depleting the size of the transcriptional effective free β-catenin pool (Fagotto et al., 1996; Sadot et al., 1998; Orsulic et al., 1999). In this setting, it has been well-characterized that reintroduction of E-cadherin into E-cadherin negative cell lines is able to decrease the transcriptional activity of beta-catenin, resulting in a reduction of cell proliferation (Gottardi et al., 2001; Perl et al., 1998; St Croix et al., 1998; Stockinger et al., 2001). Moreover, it has been clearly demonstrated that E-cadherin growth suppressive effect is dependent on a β-catenin/TCF signaling antagonism independently of the adhesive function of this protein, when reintroduced into SW480 colorectal cells (Gottardi et al., 2001). Whether this also explains E-cadherin growth inhibitory activity in normal cells or in cells that express endogenously E-cadherin and have not alterations that led to an activation of β-catenin/TCF signaling pathway has never been determined.

In order to test this hypothesis, I first analyzed the constitutive activity of this pathway in MCF-7 and HT29 epithelial cell lines grown at subconfluence using β-catenin/TCF dependent reporter assays (TOP/FOPFLASH reporter assays) (Korinek et al., 1997; Morin et al., 1997). We used the SW480 colorectal cell line as positive control for these experiments, since it holds mutations in the APC gene product and shows a high constitutive β-catenin/TCF transcriptional activity (Gottardi et al., 2001). Cells were transiently transfected with the luciferase reporter constructs TOPFLASH, which contains LEF/TCF binding consensus sequences, and the mutated FOPFLASH as control for binding specificity (Korinek et al., 1997). As shown in Figure 3A there were no constitutive activity of TOPFLASH neither in HT29 nor in MCF-7 cell lines, as compared to their relative FOPFLASH controls, and with the high constitutive transcriptional activity present in SW480 cells. These data suggest that there is not a direct relationship between E-cadherin ligation and β-catenin/TCF signaling in the control of cell proliferation, since these cells have no constitutive activity of this pathway but their growth can be inhibited when E-cadherin bonds are specifically activated onto cell surface. The lack of constitutive activity of β-catenin/TCF observed in MCF-7 is in agreement with the previously observations obtained in breast cancer cells (van de Wetering et al., 2001). However, HT29 cells present a truncation in the APC gene product. Under these conditions this protein could be able to interact with TCF-4, the only TCF member expressed in these cells (Korinek et al., 1997) and stimulate the transcriptional activity of the TOPFLASH construct. Nevertheless, in these cells a direct functional interaction with β-catenin has not been demonstrated yet using this approach.

I therefore wanted to explore the existence of β-catenin/TCF complexes in nuclear fractions from HT29 cells able to interact with TCF consensus sequences using gel retardation assays. As positive control I used nuclear extracts derived from SW480 colorectal cell line. We utilized an optimal TCF binding motif as probe, and a mutated version in the consensus sequences of this probe as control for binding specificity (Korinek et al., 1997). Specific retardation complexes were observed when used the optimal TCF probe in both HT29 and SW480 cell lines (Figure 3B). This complex was specifically supershifted by the addition of a specific antibody against TCF-4, the only TCF member expressed in these cell lines (Korinek et al., 1997; Barker et al., 1999). To test the presence of β-catenin in these retardation complexes, a monoclonal anti-β-catenin antibody was used. As shown in Figure 3B, the addition of this antibody produced the appearance of an additional band of slower mobility in SW480 but not in HT29 nuclear extracts.

This is no consistent with the previous results obtained for HT29 stably transfectant cell lines carrying the inducible expression for β-galactosidase or APC gene product (Korinek et al., 1997; Morin et al., 1997). One possible interpretation is that the transfection and isolation approaches used to obtain highly expressing clones for these cells provoked changes in the conformation and stability of β-catenin reassuring its interaction with TCF-4. Our findings demonstrate that in HT29 wild type cells and under the conditions analyzed in this study β-catenin fails to form a ternary complex with DNA. It is possible that modifications such as phosphorylation changes (Sadot et al., 2002; van Noort et al., 2002), intramolecular interactions (Miravet et al., 2002) or its association with inhibitors of β-catenin signaling like the previously characterized ICAT or duplin (Sakamoto et al., 2000; Sekiya et al., 2002; Tago et al., 2000) participate in modulating the activity observed for β-catenin in these cells. Furthermore, other possible explanation is that TCF-4 is activated by its binding to beta catenin in the cytoplasm. This interaction could activate TCF-4 and promotes its translocation to the nucleus independent of nuclear translocation of beta catenin as was previously suggested by Chan and Struhl, 2002.

The Soluble cytoplasmic Pools of β–catenin present in MCF-7 and HT29 cells are no capable to bind to E-cadherin or TCF

I decided to extend my analysis examining the levels of β-catenin in the soluble cytoplasmic pools of this cells and its potential to bind to E-cadherin or TCF, as has been previously done in Dr. Gumbiner's laboratory (Gottardi et al., 2001). To determine whether the cytoplasmic pools of beta-catenin present in these cells are competent to interact with E-cadherin or TCF, a cytosolic fraction (detergent free 100,000 supernatant) from MCF-7 and HT29 cells were subjected to consecutive affinity precipitations with a cadherin cytoplasmic domain and/or XTCF3 –GST fusion proteins. I used the colorectal SW480 cell line as a control (Figure 3C, bottom panel), since it contains transcriptionally active pools of cytosolic β-catenin able to interact to E-cadherin or TCF (Gottardi et al., 2001). In MCF-7 cells (Figure 3C, top panel), there were not detectable amounts of cytosolic β-catenin that can be precipitated neither by the cadherin cytoplasmic domain (right panel) nor by TCF –GST fusion proteins (left panel). In relation to HT29 cells (Figure 3C, middle panel), only a small amount or the cytosolic β-catenin could be precipitated by the cadherin cytoplasmic domain-GST protein and the cadherin depleted pool was not competent to interact with TCF since the cadherin depleted pool can no longer interact with GST-TCF (right panel). In the same way, using consecutive precipitations with a TCF-GST protein, only a very small fraction of cytosolic β-catenin was able to bind TCF and the TCF depleted pool is not competent to interact with E-cadherin (left panel).

The small pool of β-catenin/TCF cytosolic complexes present in this cell line was not transcriptionally active (Figure 3A), regardless to the presence of a large β-catenin cytosolic pool present in these cells explained for the existence of truncations in the APC gene product. This data strongly suggest that cytosolic stabilization of β-catenin is thus in itself not sufficient to lead transcriptionally active complexes in the cell nucleus.

The absence of transcriptionally active β-catenin/TCF complexes in these cells suggests that β-catenin/TCF signaling pathway is not involved in mediate the growth inhibitory signal triggered by E-cadherin ligation. Moreover, the existence of a large pool of the cytosolic β-catenin in all cell lines, refractory to both E-cadherin and TCF binding, not only suggests that different mechanisms may account to regulate β-catenin signaling, it also indicates that the involvement of β-catenin in the regulation of cell growth, if any, can be mediated by different signaling mechanisms present in these cells.

Direct Inhibition of β-catenin/TCF signaling pathway does Not Reduce Cell Proliferation

To analyze further whether the inhibition of cell growth directly induced by E-cadherin homophilic engagement is not linked to β-catenin/TCF signaling pathway, I decided to carry out an additional approach using two constructs that have a well-characterized role in the inhibition of β-catenin signaling at the level of target genes. The β-catenin-engrailed chimera and a dominant-negative form of TCF (Molenaar et al., 1996; Montross et al., 2000) were transiently transfected in MCF-7 cells and in SW480 control cells. Proliferating cells were labeled with BrdU and the percentage of proliferating cells after 24 h and 48 h was determined as described previously. The expression of either of two constructs significantly inhibited the number of SW480 cells in S-phase compared with mock transfected cells (Figure 3D, top panel). On the contrary, inhibition of β-catenin/TCF nuclear signaling did not have any effect in the number of MCF-7 cells in S-phase (Figure 3D, bottom panel). Thus, specific inhibition of this pathway is not able to reduce the cell growth of the MCF-7 cell line. These data together with the previous observations strongly suggests that a different mechanism may account to regulate the cell growth in the E-cadherin expressing cell lines utilized in this study, raising the interesting possibility that other or others pathways may be involved in the regulation of cell growth modulated by E-cadherin positive breast cancer cell lines.

Task 2. Identify the regions of E-cadherin cytoplasmic domain that are responsible for cell growth inhibition. (Months 18-36)

E-cadherin function depends on its association with the cytoplasmic proteins known as catenins (α-, β-/plakoglobin, and $p120^{ctn}$ catenin). This will direct me to analyze the relationship between the association of these proteins with E-cadherin and the inhibition of cell growth. $p120^{ctn}$ and β-catenin bind directly to E-cadherin. β-catenin, also acts as a bridge connecting E-cadherin to α-catenin, which in turn associates with actin filaments, directly o via α-actinin or vinculin proteins. As described above, my previous findings demonstrated that E-cadherin transduce a growth inhibitory signal to the cells through a Wnt/β-catenin signaling independent mechanism.
This suggests that other E-cadherin binding proteins such as $p120^{ctn}$ could be involved in this process.
$p120^{ctn}$ is frequently altered and/or lost in tumors of the colon, bladder, stomach, breast, prostate, lung and pancreas (Thoreson and Reynolds, 2002). In a study of invasive ductal carcinomas, p120 was completely lost in 10% of cases (Dillon et al., 1998). A second report on invasive breast carcinomas showed p120 loss in 10% of cases, 58% demonstrated heterogeneous expression and only 5% showed cytoplasmic staining (Nakopoulou et al., 2002). Recent identification and characterization of a p120-deficient colorectal cell line showed that p120 deficiency appears to result in strongly reduced levels of E-cadherin, which in turn leads to loosely organized cells that fail to maintain epithelial morphology (Ireton et al., 2002). Restoring p120 rescues the epithelial phenotype, and in light of these data, it is possible that p120 may function as a tumor suppressor through its ability to stabilize and/or regulate E-cadherin.

To investigate this possibility, different approaches will be used to analyze the role of p120ctn in the inhibition of cell growth mediated by E-cadherin engagement.
In order to specifically identify the role of p120ctn in the inhibition of cell growth, mouse genetics will be used to generate a conditional knock out in the mammary epithelia and the skin. This will provide an excellent opportunity to examine exclusively the consequences of p120 loss in vivo. The laboratory of Dr. Elaine Fuchs lab has a lot of expertise utilizing these kinds of approaches. This work will be carried out in collaboration with Dr. Albert Reynolds (Vanderbilt University, USA).

In addition, transgenic mice expressing a mutant E-cadherin that contains point mutations that block its interaction with $p120^{ctn}$ will be used to analyze the potential role of $p120^{ctn}$ in the inhibition of cell growth mediated by E-cadherin. The expression of this construct will be driven under the control of K14 promoter. This construct will be expressed in the skin and mammary epithelia. To avoid the participation of the endogenous E-cadherin protein, these transgenic mice will be mated with the E-cadherin conditional knock out in the skin and mammary epithelia, obtained from Dr. Rolf Kemler's lab (Max-Planck Institute, Germany).
Cells will be isolated from skin and mammary epithelia from both $p120^{ctn}$ knock out mice and E-cadherin-Δp120 mice. Similar studies as those described previously, such as measuring the percentage of cells in S-phase and cell proliferation after the specific engagement of E-cadherin on the cell surface, will be carried out to analyze if p120 is responsible for cell growth inhibition. If the engagement of E-cadherin in a homophilic adhesive bond is not capable of transducing a growth inhibitory signal in these cells these results will suggest that p120 could be responsible for mediating E-cadherin cell contact inhibition. Further investigation will be needed to clarify the biological mechanism by which p120 transduces the E-cadherin growth inhibitory signal.

On the contrary, if $p120^{ctn}$ protein seems not to be involved in growth inhibition, I will conclude that E-cadherin probably interacts with other growth related signals in the cells. A detailed description of the analysis of such other signals is beyond the scope of this proposal, but some directions could include the analysis of the roles of the MAPK, PKC or Ras pathways.

KEY RESEARCH ACCOMPLISHMENTS

The specific aim of this study is investigate whether the formation of E-cadherin homophilic adhesive bond itself is directly involved in the generation of growth inhibitory signals independent of other potential cell-cell interactions in primary breast cells. I am also analyzing the signaling pathways directly generated by the engagement of E-cadherin responsible for cell contact inhibition. The major findings that have resulted from this research are as follows:

1.- The specific engagement of E-cadherin in a homophilic adhesive bond is capable of transducing a growth inhibitory signal capable to reduce the proliferation of primary breast cells and certain cell lines. This process was not related with an increase in apoptosis.

2.- The growth inhibitory signal mediated by E-cadherin homophilic contacts is not mediated through a Wnt/β-catenin signaling antagonism, since:

a) Specific engagement of E-cadherin inhibits cell growth even when cells do not display β-catenin/TCF transcriptional activity.

b) The growth inhibitory signal of E-cadherin mediated contacts is not reverted by constitutively active forms of TCF.

3.- Further investigation is needed to clarify the biological mechanism by which E-cadherin transduces a growth inhibitory signal. It is known that E-cadherin function depends on its association with cytoplasmic proteins known as catenins. This will direct me to analyze the relationship between the association of E-cadherin with other catenins such as $p120^{ctn}$ in the inhibition of cell growth.

REPORTABLE OUTCOMES

Perez-Moreno M, Jamora C, Fuchs E. Sticky business: orchestrating cellular signals at adherens junctions. Cell. 2003 Feb 21;112(4):535-48. Review.

CONCLUSIONS

In this work I provide evidence that activation of E-cadherin ligation is directly involved in the inhibition of cell growth of primary breast cells. This mechanism seems to be independent of a β-catenin/TCF signaling antagonism. Whether this mechanism is directly related with other E-

cadherin-cytoplasmic domain associated proteins such as $p120^{ctn}$ is not known and requires further investigation. Nevertheless, the observed associations of cadherins and adherens junctions with signaling proteins (Yap et al., 1997; Brady-Kalnay et al., 1998; Hoschuetzky et al., 1994) raises the possibility that cadherins directly generate growth related signals. The results provided here in turn might provide an insight into the existence of other signaling and regulatory events that become activated during the activation of E-cadherin that modulate cell growth.

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APPENDICES

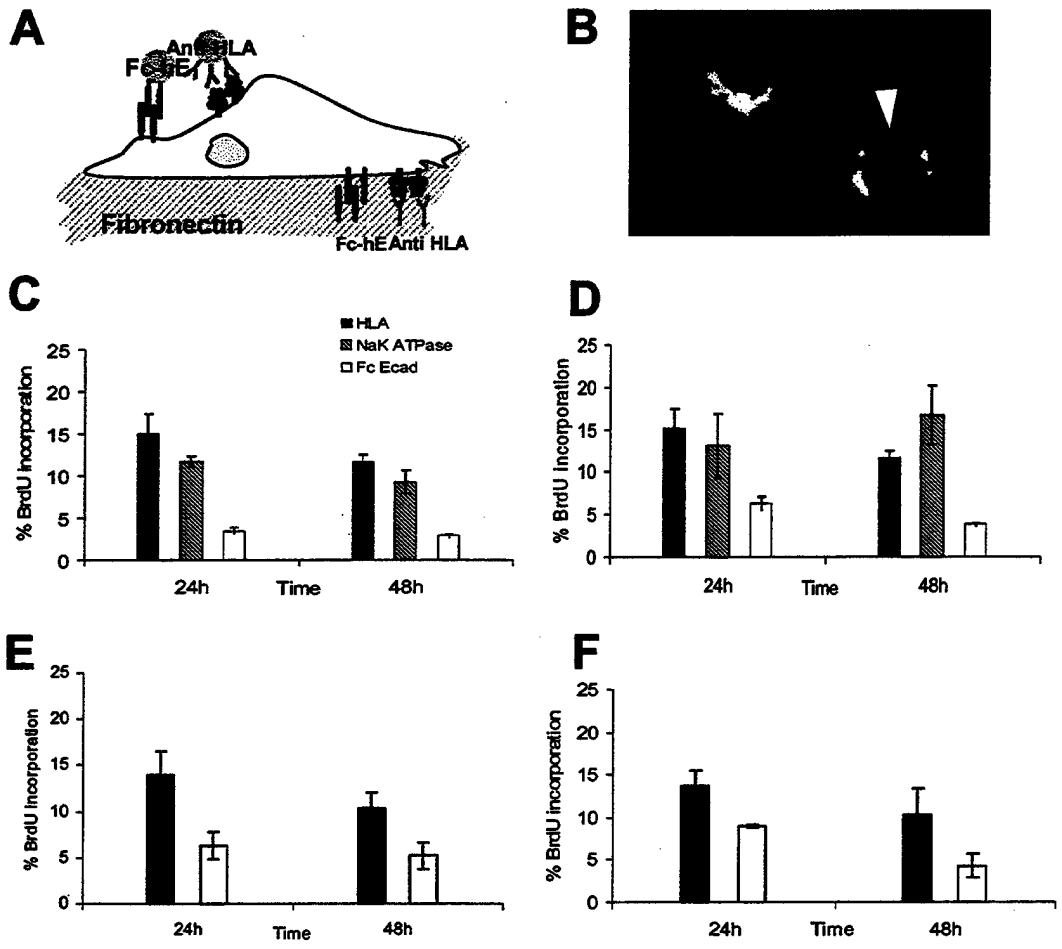

Figure 1. E-cadherin Ligation is in itself Capable to Inhibit Cell Growth of Primary Cells
(A) Schematic model of the approach used to selectively engage E-cadherin onto homophilic adhesive bonds at the cell surface. E-cadherin ligation was activated at the cell surface using Fc-hE coated microspheres or coverslips. Antibodies directed against HLA or Na+K+ATPase β1 subunit molecules were used as controls for ligand specificity. Cells were grown under conditions that stimulate cell growth from serum in the medium and from plating cells on fibronectin
(B) Binding of Fc-hE coated microspheres to the cell surface of HMEC cells plated on fibronectin. Microspheres were added in sufficient quantities to cover the cell surface of cells plated at subconfluence to avoid other types of cell-to-cell interactions. The open arrow points toward microspheres located at the cell surface level of a HMEC cell.
(C) Activation of E-cadherin ligation using Fc-hE coated microspheres or (D) coverslips triggers a strong and specific growth inhibitory signal reducing the number of HUMEC cells in S-phase as compared with ligation controls.
(E) Microspheres loaded at their medium and (F) minimum protein loading capacity with Fc-hE protein also support a robust and specific growth inhibitory signal. Data are expressed as mean ± SEM.

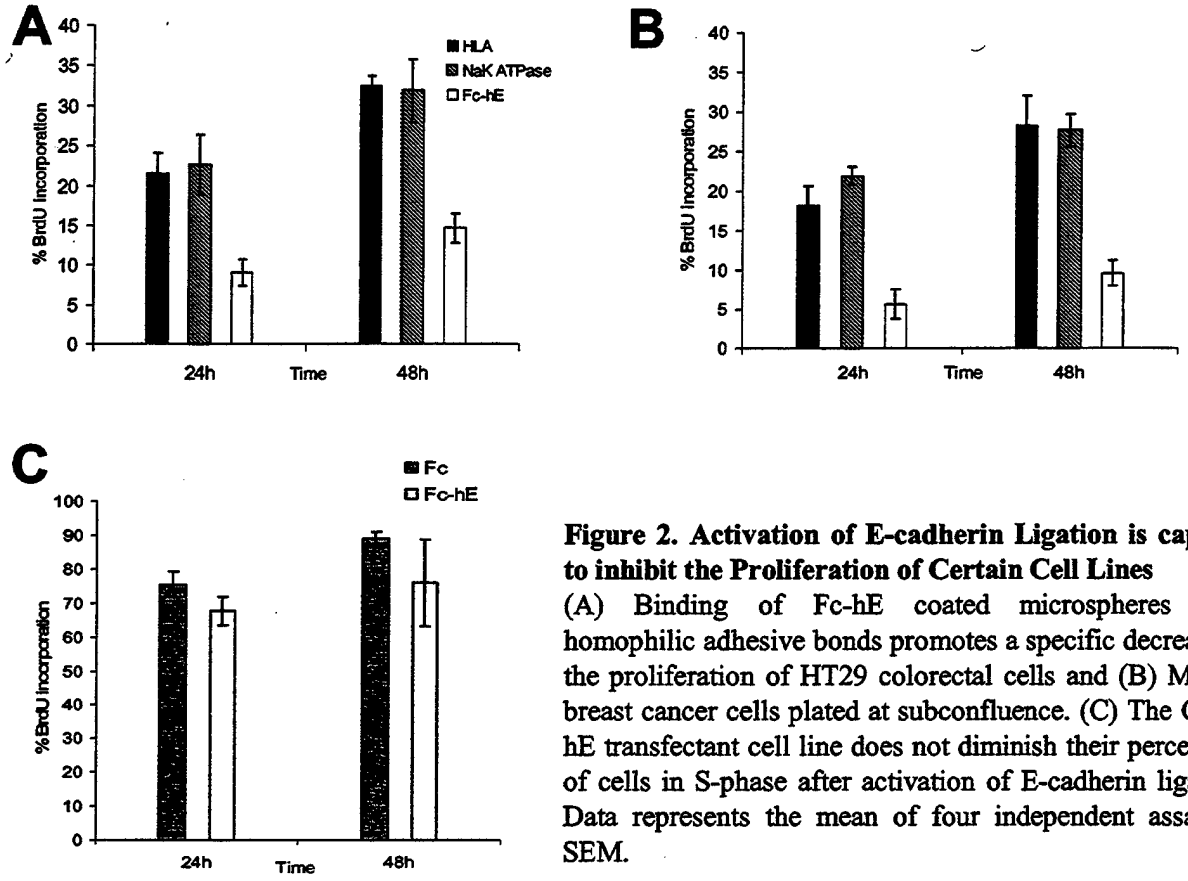

Figure 2. Activation of E-cadherin Ligation is capable to inhibit the Proliferation of Certain Cell Lines
(A) Binding of Fc-hE coated microspheres onto homophilic adhesive bonds promotes a specific decrease in the proliferation of HT29 colorectal cells and (B) MCF-7 breast cancer cells plated at subconfluence. (C) The CHO-hE transfectant cell line does not diminish their percentage of cells in S-phase after activation of E-cadherin ligation. Data represents the mean of four independent assays ± SEM.

Table I. Apoptosis rate of different cell lines after E-cadherin ligation onto homophilic adhesive bonds.

Cell Type	Time (h)	HLA	Fc-hE
HMEC	24	1.60 ± 1.50	1.08 ± 0.87
	48	1.50 ± 0.10	1.35 ± 0.15
HT29	24	1.67 ± 0.03	1.22 ± 0.08
	48	1.92 ± 0.05	1.7 ± 0.02
MCF7	24	2.40 ± 0.64	1.13 ± 0.36
	48	2.20 ± 0.05	2.19 ± 0.09

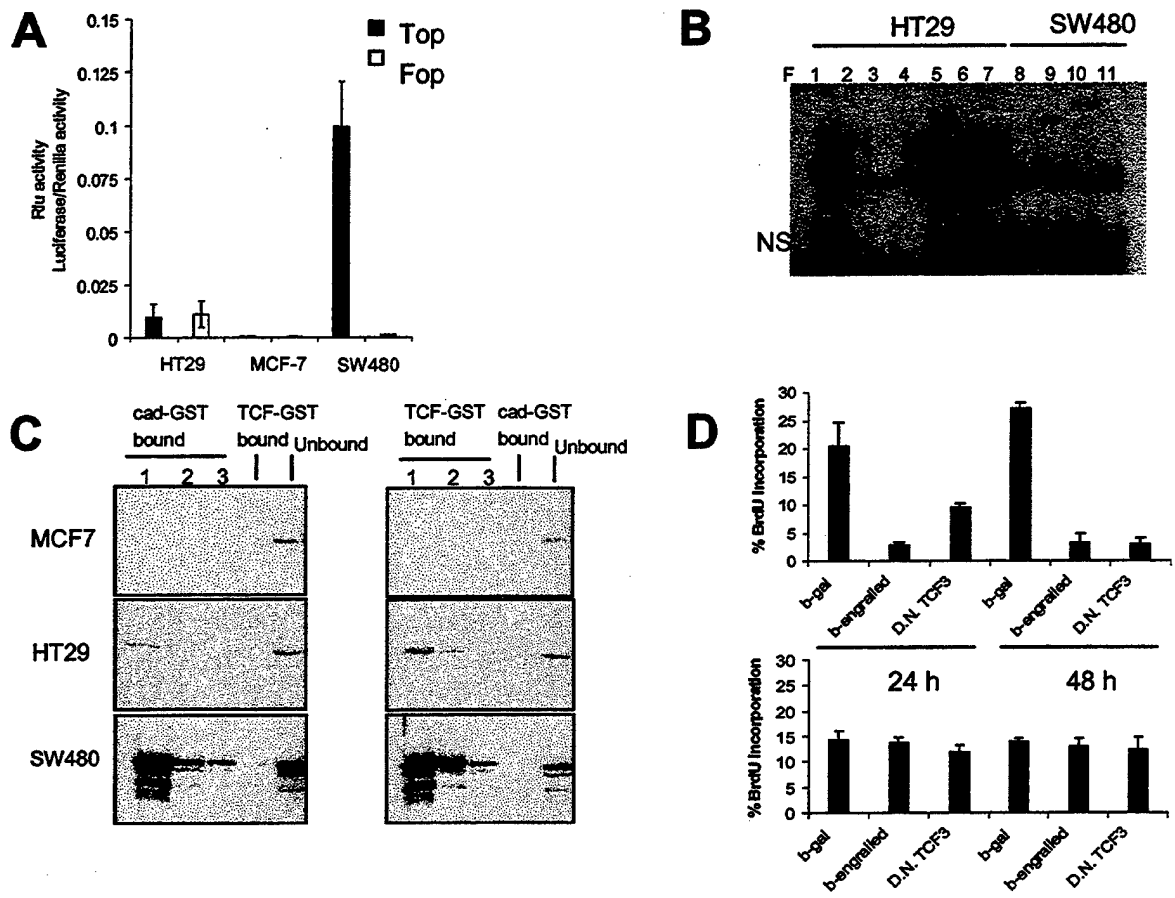

Figure 3. **Specific Activation of E-cadherin ligation Inhibits Cell Growth independent of a β-catenin/TCF signaling antagonism**
(A) Constitutive β-catenin/TCF transcriptional activity of HT29, MCF-7 and SW480 cells. Cells plated at subconfluence were transfected with either of two luciferase reporter genes TOPFLASH or mutant FOPFLASH. Renilla luciferase reporter gene was cotransfected as control for transfection efficiency. Data represents the mean of the Relative luciferase activity (RLu) of four independent assays ± SEM
(B) Gel retardation of TCF complexes of HT29 and SW480 colorectal cells. Nuclear extracts of HT29 cells and SW480 cells were analyzed in gel shift assays. 3 µg of nuclear extracts from each sample were incubated with an optimal 32P-TCF binding site probe (lanes 1, 8) in the presence of 100-fold (lane 2) and 500-fold (lane 3) molar excess of TCF cold probe, or in the presence of 500-fold molar excess of cold mutant TCF probe (lane 4). Anti-TCF4 (0.5 µg, lanes 5 and 9) or anti-β-catenin (0.5 µg, lanes 6 and 10) antibodies were added to detect the presence of TCF and β-catenin in the nuclear extracts of HT29 and SW480 cells able to bind to the optimal TCF probe. Mouse IgG was used as control for specificity (Lanes 7 and 11). Reactions were incubated for 30 min at room temperature followed by the addition of antibodies. Both HT29 and SW480 contain TCF4-specific nuclear complexes, whereas β-catenin/TCF4 complexes are just present in SW480 cells. F, free probe, NS, non-specific band.
(C) Inactive Pools of Cytosolic β-catenin in several Cell Lines (Not competent to bind Cadherin or TCF). Sequential depletion of β–catenin from a detergent free 100, 000 g cytosolic fractions of MCF-7, HT29 and SW480 cells with cadherin (left panel) and TCF-GST proteins (right panel) in vitro. Samples were separated by SDS-PAGE, and β-catenin was detected by immunoblotting. MCF-7 cells do not contain a cytosolic β–catenin pool competent to bind E-cadherin or TCF (Top panel). Only a practically undetectable fraction of cytosolic β–catenin of HT29 cells (middle panel) is competent to interact with cadherin and TCF in vitro. Furthermore, the cadherin depleted pool can no longer interact with TCF-GST and *viceversa*. SW480 cells were used as control (bottom panel). The presence of a large pool of transcriptionally inactive β–catenin was detected in all cell lines (unbound fraction).
(D) Inhibition of β–catenin/TCF transcriptional activity does not inhibit MCF-7 cell growth.
MCF-7 cells were transiently transfected with either or two myc-tagged β–catenin/TCF signaling inhibitory constructs: β–engrailed and dominant-negative TCF. (Top) SW480 were used as control for β–catenin/TCF signaling involvement in cell proliferation. β-galactosidase expressing construct was used as Mock transfection control. Transfected cells were immunodetected by immunofluorecense with a mAb to the myc-tag (clone E9.10) and proliferating cells were identified by double immunofluorescence detecting BrdU incorporation. (Bottom) The percentage of proliferating transfected MCF-7 cells after 24 h and 48 h was not decreased by the expression of β–catenin/TCF transcriptional inhibitors. Data represent the mean of four independent assays ± SEM.

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Sticky Business: Orchestrating Cellular Signals at Adherens Junctions

Review

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Cohesive sheets of epithelial cells are a fundamental feature of multicellular organisms and are largely a product of the varied functions of adherens junctions. These junctions and their cytoskeletal associations contribute heavily to the distinct shapes, polarity, spatially oriented mitotic spindle planes, and cellular movements of developing tissues. Deciphering the underlying mechanisms that govern these conserved cellular rearrangements is a prerequisite to understanding vertebrate morphogenesis.

Introduction

In order to function as a tissue, epithelial cells must have the right shape and structure to pack together with their neighbors. To undergo self-renewal while maintaining tissue anatomy, simple and stratified epithelia possess a single layer of dividing cells, orienting their mitotic spindles parallel to the underlying basement membrane (Figure 1). In stratified tissues such as the epidermis, a parallel plane of mitoses confines the transiently dividing cells to a single layer. To stratify and execute a program of terminal differentiation, cells must either rotate their mitotic plane 90 degrees and divide asymmetrically, or otherwise weaken cell substratum and cell-cell attachments to exit the basal layer, and migrate toward the skin surface. To repair a skin injury, epidermal sheets at the wound edge must move in an orchestrated manner, as occurs in developmental processes such as dorsal closure in fly embryos. During all of these processes, the exquisite cellular architecture of epithelia is achieved and maintained through dynamic permutations of protein complexes at cell-cell junctions.

In mammals, adhesion between epithelial cells is generally mediated by three types of junctions: tight junctions (TJs), adherens junctions (AJs), and desmosomes, which together constitute the Intercellular Junctional Complex (Figure 2). The complexes contain transmembrane receptors, usually glycoproteins that mediate binding at the extracellular surface and determine the specificity of the intracellular response. The associated cytoplasmic proteins of these receptors structurally link them to the cytoskeleton, thereby establishing molecular lines of communication to other cell-cell junctions and to cell-substratum junctions. The linkage of cell-cell junctions to the cytoskeleton allows single cells of an epithelial sheet to function as a coordinated tissue. Additional companion proteins connect structural and signaling elements, and thus intercellular junctions function to integrate a number of cellular processes ranging from cytoskeletal dynamics to proliferation, transcription, and differentiation.

Without diminishing the importance of other cellular junctions (reviewed in Kowalczyk et al., 1999; Tsukita et al., 2001), recent evidence has uncovered a key role for AJs not only in directing coordinated cellular organization and movements within epithelia, but also in transmitting information from the environment to the interior of cells. AJs are cadherin-dependent adhesive structures that are intricately linked to the actin microfilament network. AJs were originally identified by ultrastructural analysis, which revealed electron dense plaques of closely apposed membranes between epithelial cells. The ancient origins of AJs are likely to extend across the eukaryotic kingdom to include even single-cell organisms such as yeast. While yeast cells have no use for connecting to their neighbors, they do coordinate cytoskeletal dynamics, spindle polarity, and cell polarity, and thus employ many of the same features of AJs in multicellular organisms. During the past few years, elucidation of the assembly, functions, and dynamics of AJs have unveiled crucial roles in governing morphogenetic and patterning processes. Although the molecular and regulatory mechanisms are not fully understood, novel signaling events at AJ-cytoskeletal intersections have been discovered. These insights reveal how defects in AJs can contribute to a plethora of developmental defects and human disease.

Biochemical Organization of Cadherin/Catenin Complexes and Their Links to the Actin Cytoskeleton

The transmembrane core of AJs consists of cadherins, which cluster at sites of cell-cell contact in most solid tissues. E-cadherin, the prototype and best-characterized member of the family, is primarily expressed in epithelia. The extracellular portion of classical cadherins consists of five ectodomains, which bind calcium and adopt a rod-like template for homophilic, albeit relatively weak, interactions with E-cadherin molecules on the surface of neighboring cells. The sequential binding of proteins to the cytoplasmic tail physically bridges the cadherin receptor to the cytoskeleton and other signaling modules and results in a mature AJ (Yonemura et al., 1995; Adams and Nelson, 1998). The multiple levels of protein interaction are potential sites for the exquisite regulation of AJ complexes required during normal development.

The highly conserved, ~150 amino acid cytoplasmic tail of classical cadherins possesses a binding site for either β-catenin or γ-catenin (plakoglobin), members of the superfamily of armadillo repeat proteins (Huber and Weis, 2001; Figure 3). Binding of β-catenin's 12 repeats of 42 amino acid "armadillo" sequences to the cytoplasmic cadherin tail lends structure to the cadherin protein and is required for the transport of the newly

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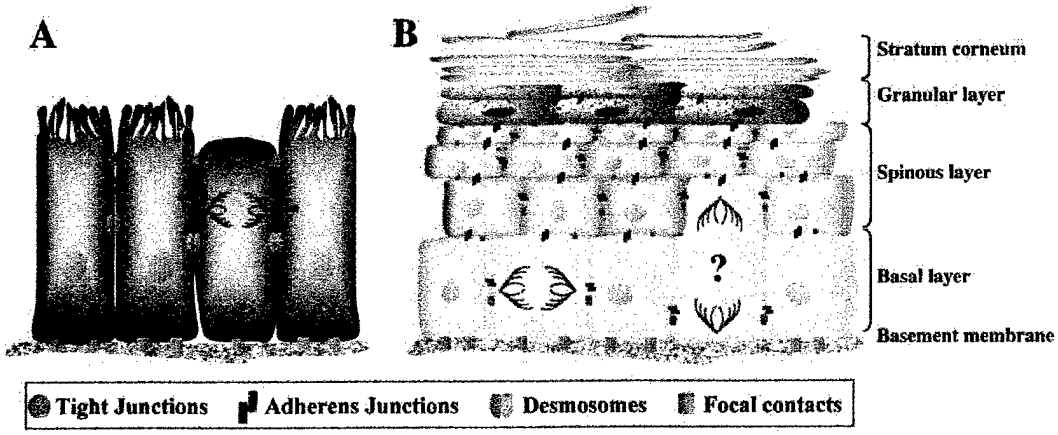

Figure 1. Organization of Simple and Stratified Epithelia

(A) Simple epithelia comprised of one layer of cells attaches to the basement membranes by focal contacts (orange squares) and to adjacent cells via adherens junctions (black rectangles) and desmosomes (pink ovals). Tight junctions (blue circles) contribute to the maintenance of apical-basolateral polarity. The plane of the mitotic spindles aligns perpendicular to the basement membrane allowing lateral expansion of the cells.

(B) The four layers of mammalian epidermis as a model of stratified squamous epithelia. Adherens junctions (black rectangles) and desmosomes (pink ovals) attach cells to each other, and integrins in focal contacts (orange squares) attach cells of the basal layer to the basement membrane. Tight junctions (blue circles) appear in the later spinous layers through the granular layer. Dividing cells of the basal layer have the spindle plane parallel to the basement membrane to allow lateral expansion of the basal layer. In embryonic skin, there are also dividing cells with the mitotic spindle plane perpendicular to the basement membrane, which allows daughter cells to contribute to the suprabasal layers. Whether this mechanism is responsible for detaching a basal cell and inducing terminal differentiation has not been unequivocally established.

synthesized E-cadherin to the plasma membrane (Chen et al., 1999). The affinity for this interaction is increased by phosphorylation of several key serine residues in the cadherin tail, and reduced by phosphorylation of β-catenin Y654, a known site of action for activated growth factor receptor tyrosine kinases (Huber and Weis, 2001). Thus, through posttranslational modifications, the strength of the AJ complex can be tailored and modified to suit the particular needs of the epithelial cell within the context of its tissue.

The ordered structure between E-cadherin and β-catenin or plakoglobin is thought to initiate an association between residues within the N-terminal head domain of the armadillo proteins and α-catenin, a protein capable of binding to F actin binding proteins. α-Catenin is normally found as a homodimer in solution, which

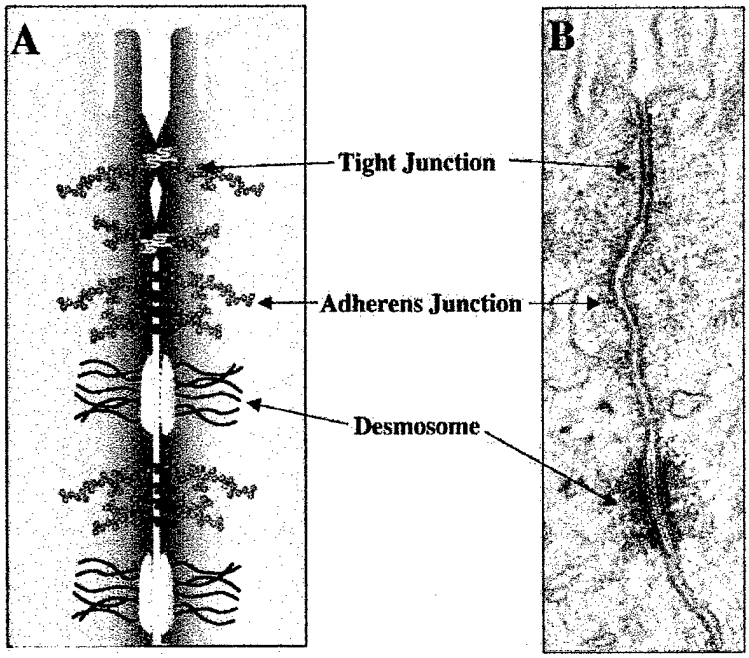

Figure 2. Composition of Three Types of Intercellular Junctions

(A) Diagram of the three major types of intercellular junctions in epithelial cells. Tight junctions are composed of transmembrane proteins linked to the actin cytoskeleton and constitute a physical barrier between the apical and basolateral regions of the cells. Adherens junctions are formed by homophilic interaction of transmembrane cadherins that are linked to the actin cytoskeleton. Desmosomes are formed by interactions between desmosomal cadherins linked to intermediate filaments.

(B) Electron micrograph depicting the ultrastructure of adherens junctions, desmosomes, and tight junctions between two murine intestinal epithelial cells (courtesy of Dr. Amalia Pasolli, The Rockefeller University).

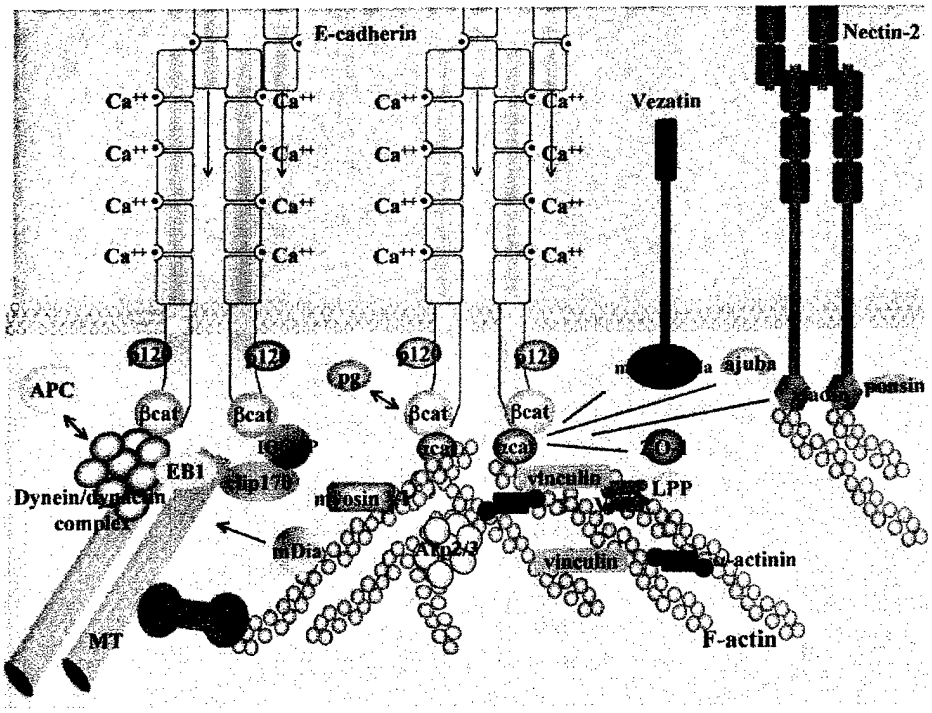

Figure 3. Protein Interactions at Adherens Junctions

E-cadherin-based adhesion junctions can associate with the actin and microtubule cytoskeletons, through associated cytoskeletal proteins. E-cadherin's direct interacting partner, β-catenin, binds to several proteins. It associates with α-catenin and links cadherin/catenin complexes to the actin cytoskeleton. Its ability to interact with some microtubule-associated proteins such as IQGAP, APC, and the dynein/dynactin complex may link E-cadherin to the microtubule network. Double arrows mean that both proteins can compete for the same site.

dissociates to bind the E-cadherin/β-catenin complex at the plasma membrane as a monomer (Koslov et al., 1997). This interaction can be modulated by the association of the E-cadherin/β-catenin complex with other proteins such as IQGAP, which blocks the binding of α-catenin to β-catenin (Kuroda et al., 1998).

α-Catenin is a central player in nucleating the assembly of a number of proteins that link E-cadherin/β-catenin complexes to F actin, a process critical not only for stabilizing intercellular junctions but also for coordinating actin dynamics at these sites (Vasioukhin et al., 2000, 2001). α-catenin can associate with F actin by direct binding through its C-terminal domain, but it can also associate directly with vinculin and zyxin family members, which in turn can bind actin and/or recruit members of the Ena/Vasp families of profilin-actin binding proteins. Biochemical studies have uncovered what appears to be a phospholipid PIP_2 (phosphatidylinositol 4,5-bisphosphate)-mediated interaction between the head and tail domains of vinculin (Johnson and Craig, 1995; Gilmore and Burridge, 1996). This head-tail interaction may block access to vinculin's Vasp and F actin binding sites, providing a potential means of controlling the association between these proteins and AJs. Finally, α-catenin can partner with the protein afadin, which also binds to F actin (Ikeda et al., 1999; Pokutta et al., 2002).

Why do AJs have so many potential binding surfaces for actin? An answer may be found in the diversity of cytoskeletal dynamics required for epithelial cells within tissues to respond to particular environmental cues. Recently, videomicroscopy of calcium-stimulated cells expressing GFP actin or GFP-cadherin has been employed to explore actin-cadherin/catenin movements during epithelial sheet formation (Adams and Nelson, 1998; Vaezi, et al., 2002; Ehrlich et al., 2002). This dynamic process starts when initial cell-cell contacts are formed by the engagement of two opposing E-cadherin/β-catenin complexes at the tips of filopodial and/or lamellopodial projections. The rate-limiting step in epithelial adhesion is the anchoring of cadherin/catenin complexes to the cortical actin cytoskeleton, promoting the clustering and stabilization of AJ proteins to form a punctum visible by fluorescence microscopy (Vasioukhin et al., 2000; Vaezi et al., 2002 and references therein). Following the appearance of this initial stable cluster of AJ proteins, additional adjacent puncta assemble, generating a zipper-like structure, which later "zips" to seal the membranes into epithelial sheets (Figure 4A; Vasioukhin et al., 2000). In vitro, the assembly and sealing of these zippers initiates near the apical surface of the polarized epithelium (Vaezi et al., 2002). A comparable situation may exist during formation of the blastoderm epithelium when AJs concentrate as spots at the apical edge of the lateral membrane and fuse into a circumferential belt during gastrulation (Tepass et al., 2002).

In epithelial cultures, a bundle of radial actin cable fibers organizes on each side of a punctum, and anchors to the underlying cortical actin ring (Figure 4A; Yonemura et al., 1995; Adams and Nelson, 1998; Vaezi et al., 2002). How the actin cables assemble is not yet fully under-

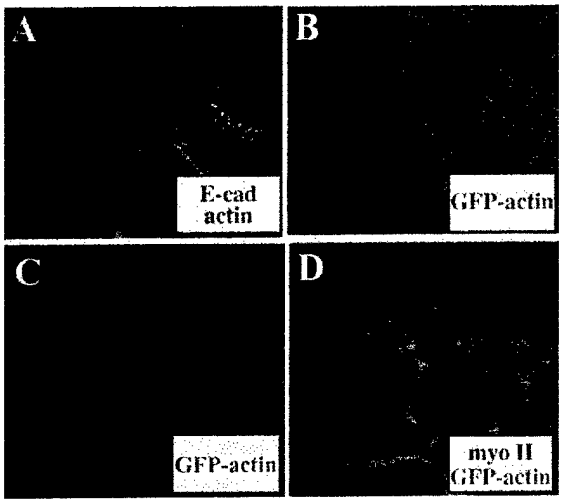

Figure 4. Actin Dynamics during Epithelial Sheet Formation

(A) At the initiation of adherens junction formation in primary mouse keratinocytes, actin-packed filopodia make contact and embed into neighboring cells. At the tips, nascent adherens junctions make contact with and attach to the underlying cortical actin cytoskeleton, forming a punctum, or stable adherens junction. This rate-limiting step increases the probability of forming additional adjacent adherens junctions, and thus the process resembles a zipper (Vasioukhin et al., 2000). E-cadherin (E-cad) is green and actin filaments are labeled with phalloidin in red.

(B) In cells expressing GFP actin, adherens junction-associated actin cables form a continuous cytoskeletal network that spans the sheet, enabling coordinated movements through the epithelium (Vaezi et al., 2002).

(C) Dorsal closure in a GFP actin expressing *Drosophila* embryo showing filopodia extending from leading edges of cells (image courtesy of W. Wood and P. Martin).

(D) The developing epidermal sheet is under tension, due to the fact that the radial actin cables (GFP actin), are linked to the central actomyosin network spanning the cell (decorated here with antibodies against anti-myosin II in red) (Vaezi et al., 2002).

stood. However, members of the zyxin and Vasp/Ena family of proteins can be observed at puncta, which are also sites of active actin polymerization (Vasioukhin et al., 2000). Recently, studies by Bear and colleagues demonstrated that Vasp can function by competing for barbed end actin-capping proteins, keeping the barbed ends open and available for extended actin polymerization (Bear et al., 2002). Although this mechanism on its own is sufficient to explain the actin polymerization seen at puncta sites, Arp2/3 complexes have also been shown to interact with E-cadherin, suggesting an underlying complexity in the dynamics (Yap and Kovacs, 2003). Irrespective of the mechanism, the outcome of actin polymerization and reorganization is the assembly of a uniform network of apical actin cables that span the entire epithelial sheet by virtue of interconnections to AJs (Figure 4B). Thus, by coordinating cytoskeletal rearrangements, individual cells can respond to stimuli as an integrated network or tissue.

Adherens junctions are also integrated into a variety of other cellular processes through associations with other types of intercellular junctions and membrane receptors. Although a detailed description of these interactions is beyond the scope of this review, it is intriguing that most epithelial sheets display closely apposed membranes where AJs alternate with desmosomes (e.g., Vasioukhin et al., 2000; Figure 2). Studies with blocking antibodies revealed that the establishment of AJs is a prerequisite for the formation of desmosomes and other junctions (Gumbiner et al., 1988). Desmosomes are specialized cadherin-mediated cell-cell junctions that attach to the intermediate filament network of keratin polymers, providing internal mechanical strength to epithelial cells (Figure 2; reviewed by Fuchs and Cleveland, 1998; Kowalczyk et al., 1999). While γ-catenin associates preferentially with desmosomal cadherins and β-catenin prefers E-cadherin, the two catenins can substitute for one another when one is missing (Bierkamp et al., 1999; Huelsken et al., 2001). Cadherin also plays an important role as a precursor for the establishment of tight junctions, which can restrict access of certain receptors and nutrients to the apical surface of the epithelium (Tsukita et al., 2001; Figure 2). Additionally, through shared interactions with afadin, AJs associate with homotypic junctions involving nectin-2, a transmembrane protein of the immunoglobulin superfamily (Ikeda et al., 1999; Takahashi et al., 1999). In fact, many additional types of membrane receptor interactions, including connexins (gap junctions), Notch and Delta, vezatin, and receptor tyrosine kinases and phosphatases, are influenced by the intimate cell-cell contacts that are directly or indirectly provided by cadherin-mediated junctions (Figure 3). In this way, AJs not only bring epithelial cells together but also affect the ability of cells to sense and respond to environmental cues.

Regulating Actin Dynamics at AJs Through the Rho Family of Small GTPases

During epithelial sheet formation and morphogenesis, actin rearrangements dramatically alter cellular architecture and motility. Members of the Rho family of small GTPases play a major role in directing actin dynamics and therefore impact profoundly upon these developmental processes. This family includes Cdc42 (which can generate filopodia), Rac (which mediates lamellipodia formation), and Rho (which promote stress fiber formation) (reviewed in Etienne-Manneville and Hall, 2002). Rho family GTPases function as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. The activation state of these proteins is finely tuned by regulatory proteins such as guanine nucleotide exchange factors (GEFs), which catalyze the exchange of GDP for GTP; GTPase activating proteins (GAPs), which increase the rate of GTP hydrolysis; and guanine dissociation inhibitors (GDIs), which inhibit the release of GDP (reviewed in Etienne-Manneville and Hall, 2002). The ability of Rho GTPases to elicit their effects during development is contingent upon their being active at the right time and place. The fact that nascent AJs are often found at the leading edges of moving cells raises the question of whether these junctions participate in the spatio-temporal regulation of Rho GTPase activity or vice versa.

Thin, filopodial protrusions of membrane filled with bundles of actin at the cell surface have been shown to mediate the formation of epithelial sheets both in vivo, such as during dorsal closure in *Drosophila* embryos,

and in vitro, during calcium-induced adhesion of mouse keratinocyte cultures. Live imaging of GFP actin during dorsal closure in vivo reveals dynamic actin-rich filopodia and lamellipodia at the leading front of the closing epithelium (Figure 4C; Wood et al., 2002; Jacinto et al., 2002), and similar dynamics are displayed in calcium-induced keratinocytes cultured from GFP actin transgenic mice (Vaezi et al., 2002; Vasioukhin et al., 2000). When filopodial protrusions are blocked by inhibiting Cdc42 activity in fly embryos, opposing epithelial sheets fail to zip or close (Jacinto et al., 2002). Conversely, adherens junction assembly seems to result in the recruitment and activation of Cdc42, as illustrated by the behavior of a GFP-tagged substrate that only binds to Cdc42 when it is in its GTP bound, i.e., active state (Kim et al., 2000). If so, it would seem that E-cadherin "primes" the cell's membrane activity, which in turn promotes AJ formation.

The ability of cadherins to influence the polarity of the cell may have its foundation in the capacity of nascent AJs to stimulate Cdc42 activation. In this regard, Cdc42 is known to promote functionality of the PAR/atypical protein kinase C (aPKC) kinase complex, which translocates to apical sites of cell-cell adhesion after calcium stimulation (Izumi et al., 1998). The PAR complex, composed of aPKC, PAR3/ASIP (aPKC specific interacting protein), and PAR6, establishes polarity in a variety of cells and tissues across the eukaryotic kingdom (Lyczak et al., 2002; Wodarz, 2002). In many epithelia, polarity requires the formation of tight junctions (TJs), which are not only adjacent to AJs, but are dependent upon AJs for their formation (Gumbiner et al., 1988). TJ formation is facilitated by a group of membrane proteins, called junctional adhesion molecules (JAMs), which recently were found to bind to members of the PAR complex (Itoh et al., 2001; Ebnet et al., 2001). These findings suggest a model whereby adherens junction formation leads to local activation of Cdc42, which in turn recruits the PAR complex, allows JAMs to bind, and promotes TJ assembly. Thus, through generation of a scaffold for the formation of tight junctions, the E-cadherin/Cdc42/PAR/aPKC pathway may facilitate the physical separation of the apical and basolateral membranes of a polarized cell (Knust and Bossinger, 2002).

Rac1 also seems to play a role in promoting AJ formation, perhaps through its ability to stimulate actin dynamics and cell-cell contacts (Eaton et al., 1995; reviewed by Braga, 2002). In cultured cells, the levels of Rac1-GTP rise following calcium-activated stimulation of cell-cell adhesion (Noren et al., 2001). Live cell microscopy with Rac1-GFP and actin-GFP proteins support this notion and reveal that Rac1 activation correlates with the extensive lamellipodia activity that is subsequently followed by stable AJ formation (Ehrlich et al., 2002; Vaezi et al., 2002). Consistent with this notion is the finding that Tiam 1 (T-lymphoma invasion and metastasis gene 1), a GEF for Rac1, localizes to lamellae and ruffles in motile cells and to sites of cell-cell adhesion in epithelial cells (Braga, 2002; Ehrlich et al., 2002; Lampugnani et al., 2002).

Precisely how the GEFs of Rac1 find their way to sites of cell-cell adhesion is not yet clear. However, initial E-cadherin engagement may be critical to the recruitment process, and in this regard, it may be relevant that homophilic E-cadherin interactions result in the activation of PI3K (reviewed in Yap and Kovacs, 2003). The activation of PI3K at developing AJs may be important for producing phosphatidylinositol lipids that in turn could serve as a localized binding platform for GEFs, such as Tiam1, that have a pleckstrin homology (PH) domain. A functional role for PI3K has been demonstrated through use of its potent inhibitor wortmannin, which blocks the recruitment of Rac1 and disrupts intercellular adhesion (Nakagawa et al., 2001).

Could activated Rac1 have roles at AJs that extend beyond lamellipodial dynamics? One possibility is that Rac might function to stimulate actin polymerization at puncta and participate in actin cable formation (Vasioukhin et al., 2000). While a role for small GTPases in this process has not yet been established, it may be relevant that a member of the Vasp family was recently shown to be recruited to filopodia through a mechanism involving activated Cdc42 (Krugmann et al., 2001). An alternative role for activated Rac1 might be to bind IQGAP, a downstream effector of both activated Cdc42 and Rac1. Rac1-IQGAP interactions might displace IQGAP from β-catenin, thereby freeing β-catenin for association with α-catenin (reviewed by Fukata and Kaibuchi, 2001). Activated Rac1 might also function to recruit rather than displace IQGAP, enabling it to perform one of its functions, such as polarizing microtubules (see below and Figure 3; Gundersen, 2002; Fukata et al., 2002).

In contrast to Cdc42 and Rac1, which seem to be recruited indirectly to AJs, Rho GTPases may partner directly with cadherin-catenin components. *Drosophila* Rho1, the homolog of mammalian RhoA, was recently found to bind to two AJ proteins: α-catenin and p120ctn (Magie et al., 2002). p120ctn is an armadillo protein that binds to the juxtanuclear region of E-cadherin, at a site that does not overlap with the β-catenin binding site (Anastasiadis and Reynolds, 2001; Braga, 2002). In mammalian cells, p120ctn appears to inhibit RhoA (Anastasiadis et al., 2000) and promote activation of Rac and Cdc42 (Noren et al., 2000). In the only functional study to date, RNAi-mediated reduction of p120ctn or α-catenin in fly embryos elicited aberrant localization of Rho1 and defects in adhesion (Magie et al., 2002). Conversely, DE-cadherin and catenin localization was disrupted in Rho1 mutant embryos, which exhibit delays in repair of epithelial wounds and dorsal closure (Figure 5; Magie et al., 2002; Bloor and Kiehart, 2002; Wood et al., 2002).

Whether the activity of Rho has a positive or negative impact upon adhesion is still controversial, but a seemingly positive role for mammalian RhoA in epithelial sheet formation and/or sheet movements has received support from several studies (reviewed by Braga, 2002). Deciphering the role of Rho in adhesion has not been straightforward, and in part this is likely to be a reflection of the many downstream targets of Rho capable of affecting actin dynamics. Several activated RhoA effectors, including PRK2/PKN kinases and the diaphanous-related formins, Dia1 and Dia2, promote cell-cell adhesion in mammalian epithelial cells (Calautti et al., 2002; Sahai and Marshall, 2002). Thus, components of AJs can regulate actin dynamics through many different mechanisms. This level of complexity seems to be required for the dramatic changes in actin organization and polymer-

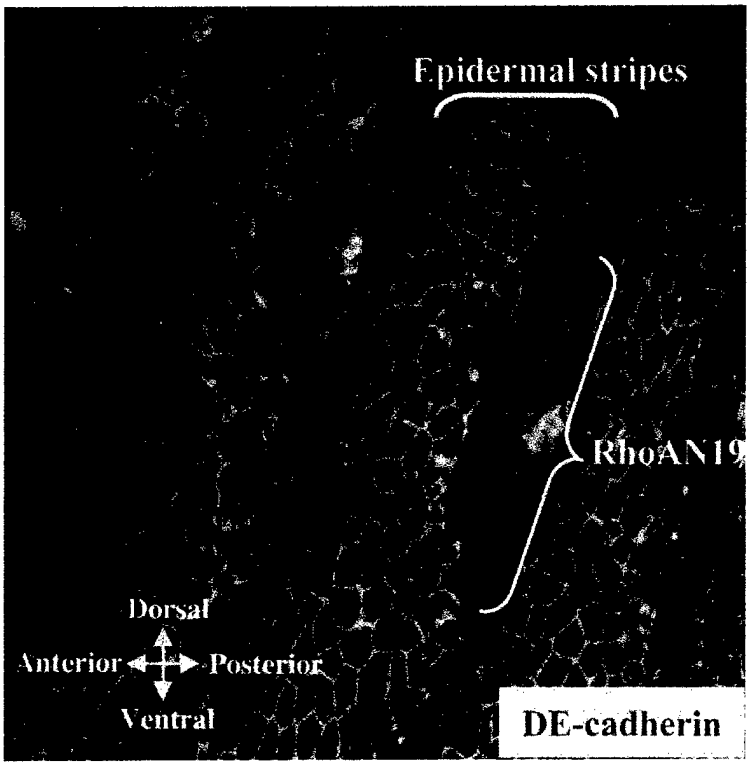

Figure 5. Effect of RhoA Mutants on E-Cadherin Expression during Dorsal Closure of *Drosophila* Embryos

Epidermal stripes of stage 15 *Drosophila* embryos expressing patches of a dominant-negative RhoA mutant (UAS-RhoAN19 under the control of enGal4). Embryos are stained with DE-cadherin antibody (green). The RhoA mutant negatively affects E-cadherin expression on patches of epidermal cells and compromises the integrity of the ventral epidermis. (Image courtesy of J. Bloor and D. Kiehart).

ization during epithelial sheet formation (Vaezi et al., 2002).

Through its indirect ability to activate myosin II, RhoA may also contribute to the generation of tension and contractile forces required for the compaction of cells into a tissue. Synchronization of these forces may be achieved by the association of AJs with the actomyosin cytoskeleton, as can be readily visualized in epithelial sheet formation in vitro (Figure 4D; Vaezi et al. 2002). Furthermore in vivo, fly zipper (zip) embryos mutant for the motor protein non-muscle myosin II, as well as RhoA mutant embryos, often fail to complete dorsal closure (Bloor and Kiehart, 2002 and references therein). During this process, actomyosin cables act as drivers of leading edge cell contractility at early stages. Later, they restrain the leading edge while maintaining a taut epithelial margin as the dorsal epithelial surfaces zip together (Kiehart et al., 2000; Jacinto et al., 2002). In *C. elegans* ectoderm, adherens junctions coordinate the actomyosin contractions that elongate the ovoid embryo into a worm (Costa et al., 1998, Priess and Hirsh, 1986). Thus, dynamic changes in cell shape and tissue movements are coordinated by the dynamic links between actomyosin cables and adherens junctions. Despite the positive roles of RhoA and myosin II, overexpression of a RhoA effector, Rho-associated kinase (Rock) or mutants in myosin light chain phosphatase are paradoxically deleterious (reviewed by Jacinto et al., 2002). Interestingly, in mammalian cells, inhibitors of Rock relax the tension across developing epithelial sheets and accelerate membrane sealing (Sahai and Marshall, 2002), but they also adversely affect the ability of sheets to generate the radial actin cables and coordinate cellular movements (Vaezi et al., 2002). Taken together, these findings suggest the importance of striking the right balance of tension and adhesion in epithelial sheet movements and tissue formation.

A Link Between AJs and Spindle Polarity: Parallels Between Yeast Buds and Adherens Junctions

Unlike actin filaments, microtubules are not required for AJ assembly; however, they do physically associate with adhesive structures. The molecules responsible for the connection between microtubules and AJs are not yet clear. One possible candidate is APC (adenomatous polyposis coli), which binds to β-catenin as well as to the microtubule binding protein EB-1 (Berrueta et al., 1998; Askham et al., 2002). Another potential player is Clip-170, which binds to microtubules as well as to IQGAP (Gundersen, 2002). The actin binding protein ACF-7 localizes to the tips of microtubules at the leading edge of migrating cells, and in response to calcium, it reorganizes with microtubules to sites of cell-cell adhesion (Figure 3; Karakesisoglou et al., 2000). ACF-7 is a unique candidate for directly integrating a microtubule-actin-AJ connection, as it possesses binding sites for both actin filaments and microtubules (Karakesisoglou et al., 2000; Sun et al., 2001). Given its large size (>600 kDa) and localization, ACF-7 may also bind to other proteins, such as dynein-dynactin patches, which form at sites along the actin cortex near developing cell-cell contacts (Figure 3). In this regard, it is interesting that another microtubule binding protein, the motor protein dynein, is not only an organizer of dynein-dynactin patches, but also binds to β-catenin (Ligon et al., 2001).

These putative connections between microtubules

and AJs are particularly fascinating in light of recent evidence that implicates adherens junctions in symmetric and asymmetric cell divisions. The orientation of mitotic spindle during cell division is critical in determining the organization and architecture of cells within epithelial tissues. Whether simple or stratified, epithelia often need to maintain a single layer of symmetrically dividing cells anchored to an underlying basement membrane (see Figure 1). To do so, an expanding epithelium must orient its spindles parallel to the basement membrane. Often in development, however, cells shift their spindle along the apical-basal axis, such that only one daughter cell remains within the plane. Such asymmetric divisions may be able to generate stratified epithelia, or produce new cell types, such as in the formation of a hair follicle (Byrne et al., 1994).

How cells choose their axis of division has been a matter of intense investigation, and recently, AJs have emerged as essential components of the machinery. Lu et al. (2001) discovered that disruption of the adherens junction-associated component E-APC and its binding partner EB-1 in *Drosophila* results in the conversion of symmetric epithelial divisions to asymmetric ones during embryogenesis. Tissue culture studies of EB-1 RNAi-treated cells suggest that this phenotype may result from malformed mitotic spindles, defocused spindle poles, and mispositioned spindles away from the cell center (Rogers et al., 2002).

A survey of the proteins involved in spindle orientation in the single-cell yeast *Saccharomyces cerevisiae* reveals some striking parallels with the cytoskeletal-associated proteins that interact with AJs. In budding yeast, EB1 is a genetic determinant of spindle orientation, which is established through capture of astral microtubules and tethering along the mother-bud axis. Dynein-dynactin complexes are also involved later in this process by maintaining spindle orientation and facilitating spindle movement during mitosis (Theesfeld et al., 1999; Heil-Chapdelaine et al., 2000). The bud site, sometimes referred to as the polarisome (Sagot et al., 2002), contains attachment sites for the astral microtubules (Figure 6). EB1, dynein-dynactin and other microtubule-associated proteins, including Kar9 (Miller and Rose, 1998), a possible APC homolog, contribute to docking the microtubules to the cortical actin and then stabilizing the interaction. Although no ACF7-like homolog has been found in yeast that can directly link together actin and microtubules, many of the other functional homologs involved in the basic process of spindle orientation in unicellular organisms seem to be shared with AJs (Figure 6; compare with Figure 3).

The parallels between AJs and bud sites can be taken one step further, to look at the similarities in actin dynamics and polarized growth. Bud-associated actin cables appear to initiate and grow from the bud along the mother-bud axis (Yang and Pon, 2002). These cables are thought to serve as a polarizing highway for the directional transport of both proteins and RNAs, a process that may also involve the myosin Myo2p (Pruyne et al., 1998). Moreover, they may function together with the cortical basket of actin cables in the mother cell to help guide the astral microtubules into the bud (Theesfeld et al., 1999; Yin et al., 2000). Similarly in mammalian cells, the radial actin cables linked to AJ appear to utilize

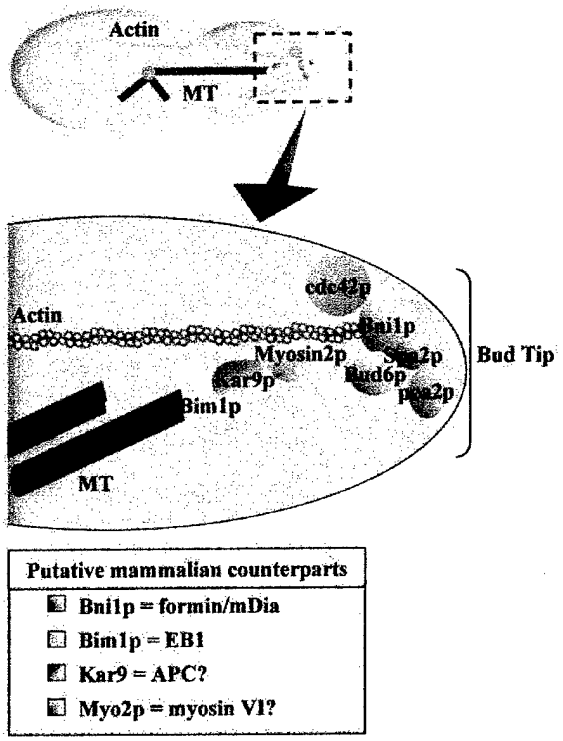

Figure 6. Proteins Associated with Capturing Actin and Microtubules at the Bud Tips of Yeast

The top image shows how both the actin and microtubule cytoskeletons of the mother cell extend into the daughter cell and converge at the bud tip. The middle image shows a magnified view of the bud tip to illustrate the complement of proteins implicated in nucleating actin cable formation at the bud tip and in capturing microtubules at this site. Bud6p, Spa2p, Pea2p and Sph1p, which determine the bud tip site, are distinct from the cadherins and catenins, which initiate the formation of adherens junctions. However, there are marked parallels in the cytoskeletal dynamics and in the associated proteins that are involved at these tips in budding yeast and at adherens junctions in mammalian cells. The table lists possible mammalian counterparts of the yeast proteins.

myosins, in both stabilizing cell-cell adhesion and promoting cellular polarization (Vaezi et al., 2002; Geisbrecht and Montell, 2002). Finally, actin polymerization and cable dynamics at bud sites rely upon the Rho-GTP activated formin Bni1p in yeast. If parallels to AJs hold, this offers a possible function for Rho-GTP and mDia1 at cell-cell junctions in multicellular organisms (Figures 3 and 6; Sahai and Marshall, 2002). While additional work is necessary to truly establish the functional equivalence between a number of these yeast and mammalian proteins, the parallels suggest a tantalizing evolutionary link between the two systems.

AJs and Cell Sorting during Development

During embryogenesis, boundaries often develop between morphologically homogeneous cell populations. It has long been surmised that differential cell affinities orchestrate the formation of tissue boundaries, and cadherins play a central role in this process. Mammalian cadherins now encompass a superfamily of >20 proteins, which are differentially expressed in elaborate pat-

terns. In a now classic experiment, Takeichi and coworkers transfected two of them, E- and P-cadherin, into separate groups of L cells, which normally possess little or no cadherin activity (Nose et al., 1988). The transfected cells preferentially adhered to cells expressing the same cadherin subclass and they developed epithelial sheets. In contrast, untransfected cells associated with mesenchymal cells, which do not express cadherins.

Recent findings suggest that additional transmembrane receptors, particularly the ephrin (Eph) receptor tyrosine kinases, contribute to the sorting specificity of cell populations (reviewed by Kullander and Klein, 2002). Eph receptors comprise a family of receptor tyrosine kinases whose ephrin ligands are also membrane bound. Receptor-ligand interaction and signaling requires direct cell-cell contact, and recently, the function of several Eph receptors and ephrins has been found to depend upon E-cadherin and cytoskeletal dynamics. Thus, in non-epithelial cells, ectopic expression of E-cadherin can induce EphA2 receptor expression, and in epithelial cells, AJs regulate localization of the protein (Orsulic and Kemler, 2000).

Like the cadherins, Eph receptors are expressed in complex patterns during embryonic and postnatal development. However, in contrast to cadherins, Ephr-Eph associations can mediate repulsion or adhesion, depending upon the developmental context (Kullander and Klein, 2002). A particularly intriguing example of this is the EphA7 receptor, which functions with its ligand ephrinA5 in early neural tube closure. An alternatively spliced mRNA encoding a truncated version of EphA7 interferes with EphA7's ability to act in repulsion and instead promotes adhesion (Holmberg et al., 2000). Gene-targeting studies reveal that in the absence of ephrin ligands or receptors, cells otherwise positioned at one place in a tissue now relocalize to distinct sites (Batlle et al., 2002; Kullander and Klein, 2002). Thus, tissue boundaries are sometimes established at interfaces where Eph receptor-expressing cells meet ephrin ligand-presenting cells, reflective of a role for repulsion. Within the confines of these tissues, adhesive forces are critical. While the mechanisms underlying these processes are just beginning to emerge, it seems likely that both cadherin-mediated adhesive affinities and Eph receptors and their ligands will be important in defining and maintaining sorting behavior and boundaries, and in determining positioning, migration, and differentiation within tissues.

Adherens Junctions, Stem Cells, and Early Cell Specification

Recently, cadherins and their close associates have been implicated in providing spatial cues to stem cells. Whether in early development or in adult tissues, stem cells reside in customized niches or microenvironments that contribute to their unique ability to divide asymmetrically to give rise to self and to a daughter with distinct properties. An interesting example of cadherin regulation of stem cells comes from studies on the *Drosophila* ovary (Song et al., 2002). Germ stem cells (GSCs) reside in a niche that is established by the interaction of stem cells with their basement membrane (extracellular matrix) and with neighboring differentiated cells. An asymmetric division of a germ stem cell causes the physical dissociation of one of its daughters from this specialized environment, depriving it of the self-renewing signals and promoting its differentiation. When DE-cadherin is reduced or absent in the *Drosophila* germarium, GSCs no longer interact with the 5–6 cap cells of the ovarian niche, and they differentiate prematurely. How universal is a role for cadherins in maintaining stem cells in their niche, and are they simply the glue that keeps the cell in its microenvironment? Research on sensory organ development and neuroepithelial cell division suggests that AJs may in fact play an active role, by influencing the ability of multipotent cells to divide asymmetrically.

To initiate asymmetric cell divisions, neural precursors of the sensory organ and CNS utilize a planar polarity and an apical-basal polarity cue respectively, regulated by the protein Bazooka, the *Drosophila* homolog of Par3 (Lu et al., 2001). For example in the *Drosophila* CNS, the neural progenitors cells called neuroblasts originate from neuroepithelial cells, which are polarized along the apical-basal axis and divide symmetrically along the planar axis (Figure 7A). In a process involving Notch signaling, neuroblasts delaminate from the neuroectoderm and divide asymmetrically along the apical-basal axis (Jan and Jan, 2001). Some relevant changes associated with this are the loss of cell-cell contacts and the redistribution of proteins required for asymmetric division. The protein Bazooka localizes to the apical membrane and the proteins Pon and Numb to the basal membrane. The expression of inscuteable and its apical targeting through interaction with Bazooka leads to activation of apical-basal spindle cues (Figure 7B; Jan and Jan, 2001). As opposed to neuroblasts, neuroepithelial cells divide symmetrically along the planar axis and segregate Bazooka, Pon, and Numb equally between the two daughter cells. This suggests that other polarity cues may prevent asymmetric division.

What are the molecular cues that cause the spindle plane to rotate and the asymmetric divisions to begin? At present there is no definitive answer, but genetic approaches have suggested that inscuteable is required for asymmetric cell divisions (Kraut et al., 1996; Schober et al., 1999; Wodarz et al., 1999). This said, in a recent study by Rath et al. (2002), some neuroblast lineages were identified that divide asymmetrically in the absence of this gene. The studies of Lu et al. (2001) indicate that when *Drosophila* embryos are treated with RNAi to diminish EB1 or APC levels, the ectodermal cells no longer maintain their polarity, and their divisions become asymmetric and misoriented (Figure 7C). These studies did not involve direct disruption of core adherens junction proteins, and hence it cannot be judged from these studies alone that AJs can override the apical-basal apparatus for spindle positioning. In this context, it is important to consider studies on sensory organ development, where a partial loss of DE-cadherin function and expression of a dominant-negative resulted in defects in the orientation of certain planar asymmetric cell divisions as well as the positioning of Bazooka (Le Borgne et al., 2002).

Taken together, the studies are consistent with a model whereby epithelial cells may utilize two competing sets of polarity cues for spindle positioning: a lateral

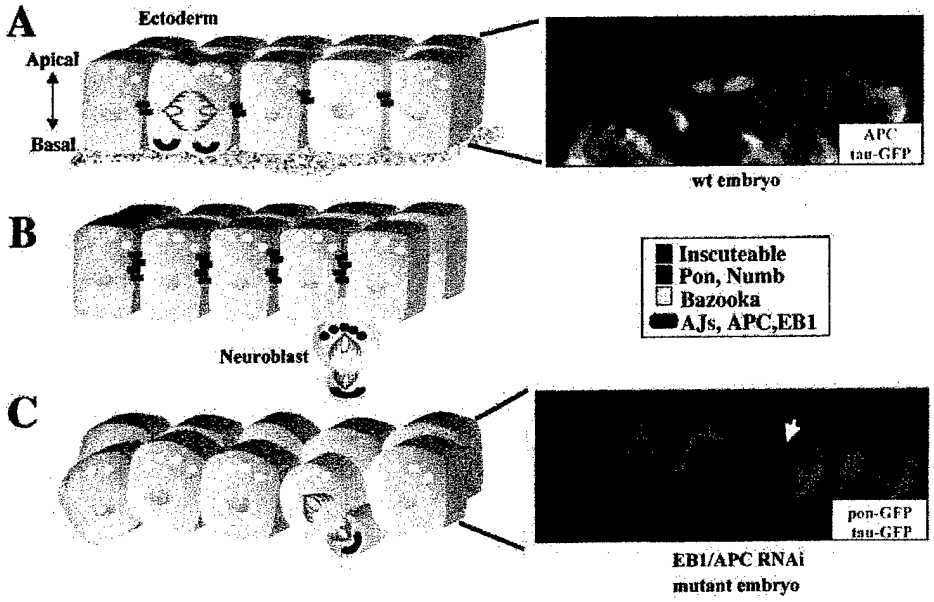

Figure 7. Possible Role for Adherens Junctions in Spindle Orientation

(A) Left image illustrates a symmetrical cell division within the plane of the embryonic ectoderm, which is polarized due to maintenance of adherens junctions and attachment to an underlying extracellular matrix. Bazooka (yellow dots) localizes along the apical membrane of the epithelia while Pons and Numb (blue crescent) localize along the basal membrane. The orange cell depicts a neural-competent ectodermal cell, which will develop into a neuroblast as a consequence of Notch/delta signaling within the ectoderm. Right image shows symmetric division and anterior-posterior spindle orientation in the ectoderm of *Drosophila* embryo expressing a tau-GFP fusion protein to highlight the spindle (Lu et al., 2001). Intercellular contacts are highlighted with antibodies to APC (red), which associates with adherens junctions.

(B) Left image. When a neuroblast develops, and exits the ectoderm, it acquires the inscuteable protein (black dots), which associates with Bazooka, and which is likely to be involved in inducing asymmetric divisions along the apical-basal plane at least in some neural lineages. Adherens junctions are also lost as the cell breaks contacts with its neighbors. Recent evidence suggests that this loss may also contribute to asymmetric cell divisions.

(C) When *Drosophila* ectoderm expresses RNAi for EB1 or APC, adherens junctions and cell polarity is disrupted, and asymmetric divisions are seen (Lu et al., 2001). Shown at right is a section from a pon-GFP, tau-GFP embryo with reduced APC, illustrating the skewed orientation of the mitotic spindle and the enhanced crescent of Pon at the base, where one pole of the spindle is attached (courtesy of F. Roegiers, B. Lu, and Y.N. Jan).

polarity cue mediated by AJs and/or the proteins that it recruits, and an apical-basal polarity cue regulated by Bazooka. If levels of adherens junction proteins are high, a lateral cue would be expected to prevail and cells would divide symmetrically, within the lateral plane. If the levels of AJ proteins are reduced and/or the apical-basal cues are accentuated (e.g., by activator proteins such as inscuteable), then the apical-basal polarity cue might be dominant, inducing asymmetrical divisions and resulting in a cell's exit from its surroundings (Jan and Jan, 2001). This model is tantalizing, as there are many developmental processes such as stem cell activation and differentiation, where changes in cadherin expression and adherens junction dynamics have been observed, at times when rotations in spindle orientation must also be established. Future studies will determine the extent to which adherens junction-cytoskeletal dynamics might be able to overpower the apical-basal cue for asymmetric division.

Several twists on this theme come from the possibility that Bazooka can influence adherens junction formation and this could have a direct impact on the directionality of asymmetric divisions (Lu et al., 2001; Bilder et al., 2003). Another protein that can impact this process is Rap1, which regulates the localization of components of AJs at the apical side of the epithelium of the *Drosophila* wing (Knox and Brown, 2002). The defects in cell shape and morphogenesis of *Drosophila* embryos seen in Rap1 mutants are consistent with the notion that the positioning of adherens junction could play a role in cell mobility and division. Though speculative, such mechanisms could provide a molecular explanation of how each daughter cell in sensory organ development maintains the same asymmetric division as its mother cell (Le Borgne et al., 2002).

One final note is that like the adherens junction proteins, the apical-basal spindle polarity proteins that are membrane-associated seem to have no counterparts in yeast. It seems to be the cytoskeletal dynamics associated with these junctions, rather than the membrane-associated proteins per se, that are conserved.

Regulating Cytoskeletal-AJ Connections and Downregulating Cadherins

Adherens junctions and their associated cytoskeletons must be dynamic to accommodate the tremendous degree of intercellular remodeling that occurs during morphogenesis, tissue homeostasis, and recovery from injury. With a possible role for cadherin levels in regulating such critical processes as spindle positioning, epithelial

sheet movements, and intercellular adhesion, increasing interest has been placed upon how the levels of cadherins and their associates are controlled. Not surprisingly, a number of mechanisms have been implicated, and the regulation appears to be complex and finely tuned.

We have already alluded to the posttranslational modification of Y654 on β-catenin that decreases its affinity to E-cadherin, thereby weakening the stability of AJs (Huber and Weis, 2001). Following this tyrosine kinase-activated modification, the disassembled E-cadherin complexes are subject to endocytosis and ubiquitination-mediated degradation (Fujita et al., 2002). Tyrosine phosphorylation of β-catenin influences a wide variety of developmental processes (Dumstrei et al., 2002 and references therein). In tumorigenesis, where tyrosine phosphorylation levels are unnaturally elevated, excessive β-catenin phosphorylation is accompanied by increased invasiveness (reviewed by Gumbiner, 2000). Taken together, these findings underscore an important and intimate link between growth factor signaling and control of cell-cell contact stability. Like β-catenin, cadherins can also be phosphorylated by tyrosine kinases. Recently, an E3 ligase called Hakai was shown to bind to E-cadherin in a phosphotyrosine-dependent manner causing the shuttling of internalized E-cadherin to the lysosome rather than recycling it back to the plasma membrane (Fujita et al., 2002). Thus, Hakai has the ability to regulate adhesion by modulating the amount of cell surface cadherin.

Interestingly, if the disassembled cadherin-catenin proteins meet the action of tyrosine phosphatases (PTPs) prior to ubiquitination, they are spared, and intercellular adhesion can be restored (Gumbiner, 2000). An example of this is the recent discovery of a PTP that coprecipitates with the endothelial VE-cadherin and reverses its phosphorylation by the tyrosine kinase receptor for VEGF (Nawroth et al., 2002). One possibility is that VEGF-receptor action may loosen cell-cell contacts to modulate transendothelial permeability and to allow blood vessel sprouting and migration during angiogenesis, a response which PTP action might downregulate. However, when endothelial cells are subjected to shear stress, they rapidly anchor both VEGFR-2 and VE-cadherin to the endothelial cytoskeleton, promoting their association and the transduction of shear-stress signals. In this regulatory twist, VE-cadherin's role seems to be critical, as cells lacking this cadherin cannot transduce the signals (Shay-Salit et al., 2002). Additionally, without VE-cadherin, VEGF-mediated cell survival and angiogenesis are compromised. Based upon this example, AJs are likely to play pivotal roles in regulating cellular responses to growth factors and other environmental signals in specific cellular hierarchies during development.

In addition to being direct targets for certain tyrosine kinases, cadherins also interact with a number of other proteins such as p120ctn and Rho GTPases (described above), that can influence their activity and stability. The protein p120ctn has been shown to act both as a positive and negative regulator of cadherin adhesiveness (Anastasiadis and Reynolds, 2001). Nevertheless, recent evidence using a p120ctn-deficient colorectal cell line indicated a crucial role for p120-E-cadherin interaction not only for the proper localization and function of E-cadherin but also an increase of protein expression (Ireton et al., 2002). Another intriguing potential partner for E-cadherin is the presenilin 1 (PS1) protein involved in Alzheimer's disease. Several reports suggest that PS1 directly binds to E-cadherin, although whether this association stabilizes E-cadherin (Baki et al., 2001) or targets E-cadherin for cleavage and AJ disassembly (Marambaud et al., 2002) is not yet clear. Additionally, PS1 facilitates the stepwise phosphorylation of β-catenin that targets it for degradation, and conversely, loss of PS1 leads to stabilization of β-catenin, enabling it to function outside the realm of AJs (Kang et al., 2002). While the implications for Alzheimer's disease remain unknown, PS1 is broadly expressed in epithelia, and these recent findings suggest that its regulation may impact on AJ-cytoskeletal dynamics.

Intricate regulation of the transcription of the E-cadherin gene bestows an added level of sensitivity for controlling E-cadherin levels in tissue morphogenesis. The best-studied element of the E-cadherin promoter is an E-box which binds factors such as Snail, Slug, E12/E47, and SIP to promote transcriptional repression of the E-cadherin gene (Bolos et al., 2003 and references therein). Genetic ablation of snail in mice results in early and striking embryonic abnormalities, including the development of a mesoderm with epithelial characteristics such as AJs and apical-basal polarity (Carver et al., 2001). Conversely, overexpression of snail and its cousin slug result in epithelial to mesenchymal transitions (EMTs) in vitro (Bolos et al., 2003 and references therein). EMTs play a broad role in the normal development of tissues, including kidney and skeletal muscle. Snail has also been implicated in specifying mesodermal fate during gastrulation (Carver et al., 2001, Ciruna and Rossant, 2001). A variety of epithelial cancers show elevation of these repressor proteins, and this correlates with tumor invasiveness (Comijn et al., 2001 and references therein). It will be interesting in the future to see whether changes in the levels of these proteins might influence developmental processes such as epithelial budding or branching morphogenesis, where localized downregulation in adhesive interactions may be required to remodel epithelial junctions.

Direct Participation of Cadherin-Catenins in Signaling Pathways: Adherens Junctions and Beyond

Given the multiple roles of AJs in morphogenesis, it would not be surprising to find communication between AJs and the nucleus. Although the evidence is not yet conclusive, one possible communicating line could be through β-catenin. For nearly 10 years, it was difficult to reconcile that while in *Drosophila* β-catenin was known to be a component of the wingless signal transduction pathway involved in segment polarity, its only known mammalian counterpart at the time, plakoglobin, was a component of intercellular adhesion. When β-catenin was discovered to interact with a new partner, the DNA binding protein Lef-1 (Behrens et al., 1996), the *Drosophila* relative pangolin was quickly placed genetically in the canonical Wnt/Wingless pathway. β-catenin had a newfound role as a transcriptional regulatory protein in both systems (reviewed by van Noort and Clevers, 2002).

Early on, it was recognized that canonical Wnt signaling prevents any excess β-catenin not utilized in AJs from being targeted for ubiquitination and degradation via the proteosome pathway (reviewed by Moon et al., 2002). This stabilization of cytosolic β-catenin allows for the direct interaction of β-catenin with transcription factors of the Lef/Tcf family (Moon et al., 2002). Remarkably, despite little or no sequence identity between E-cadherin and these DNA binding proteins, they both bind to the same site on β-catenin (reviewed by Pokutta and Weis, 2002). Exactly how β-catenin acts to regulate Lef/Tcf activity is not yet clear. In *Drosophila*, Pangolin acts as a repressor, and recent transgenic evidence suggests that Armadillo (β-catenin) might function by exporting Pangolin out of the nucleus to relieve repression and activate downstream target genes (Chan and Struhl, 2002). In mammalian cells, however, Lef1/Tcf proteins often act to transactivate rather than repress genes and they often concentrate in the nucleus of cells upon receipt of a Wnt signal (Merrill et al., 2001 and references therein). In addition, in an in vitro assay with chromatin templates, recombinant β-catenin strongly enhanced binding and transactivation by Lef-1 (Tutter et al., 2001). Taken together, these findings suggest that β-catenin may function both in chromatin remodeling and in nuclear export. As the many different interacting partners of β-catenin are elucidated, the complex mechanisms involved in its actions should become clearer.

While the precise mechanism underlying β-catenin's link to Wnt-mediated transcription remains controversial, the pathway is utilized broadly in development and there is widespread agreement that the consequences of excessive Wnt signaling and constitutive stabilization of β-catenin are frequently tumors and cancers. In this regard, β-catenin differs from E-cadherin and α-catenin, which are mutated or downregulated in a number of epithelial cancers (Conacci-Sorrell et al., 2002). However, it could be that downregulation of E-cadherin could free β-catenin to participate in transcriptional regulation. Since β-catenin's binding sites for E-cadherin and Lef1/Tcf are shared, the level of E-cadherin in cells is likely to impact significantly on the amount of β-catenin that is available for Lef1/Tcf (Gottardi et al., 2001). Conversely, activation of Wnt signaling results in stabilized β-catenin that might directly act on Lef1/Tcf complexes to transcriptionally downregulate key adhesion genes such as E-cadherin or α-catenin. Some evidence for this exists in the mouse brain, where a correlation between Wnt 1 signaling and repressing E-cadherin mRNA expression has been reported (Shimamura et al., 1994).

Conclusions

In closing, epithelial cells have an amazing ability to simultaneously change their shape, polarity, transcriptional agenda, and proliferation status, and they can move through tissues with intricate precision during development and differentiation. AJs appear to be at the crossroads of morphogenetic and patterning processes in tissues that are dependent upon intercellular connections for their development. The molecular mechanisms involved in morphogenesis are not yet well-defined, and much remains to be done to understand how external signals are transmitted through AJs and their neighboring receptors to the cytoskeleton in order to communicate this information to other critical systems within the cell.

While the details are often still fuzzy, the emerging picture suggests that the levels of AJ proteins in cells are central to the fate the cells will adopt. Elevated levels of cadherins may be key determinants in distinguishing epithelial cells from mesenchymal cells, and in establishing and maintaining the proper polarity and spindle orientation of cells within a tissue. Downregulation of cadherin expression during development or differentiation may unmask underlying mechanisms controlling spindle orientation that promote asymmetric cell divisions, a key process in stem cell determination. When downregulation of cadherin expression happens unnaturally, tumor progression and invasion are often a consequence. Finally, complete loss of cadherin results in apoptosis and tissue necrosis (Boussadia et al., 2002). Superimposed on the importance of cadherin levels are the levels of its close associates β-catenin and α-catenin, which expand the repertoire of cellular responses by integrating cytoskeletal networks and transcriptional regulation (van Noort and Clevers, 2002).

With the advancement of genome analyses, the field has been informed by studies encompassing the eukaryotic kingdom, extending even to yeast, which has no need for coordinated cell movements and behavior, but which does need to orient the spindle and control polarity. With advancements in microscopic techniques, it has become possible to monitor adherens junction and cytoskeletal dynamics in living cells and tissues. Thus, many of the tools are now available to tackle the complex process of morphogenesis, a difficult task that until recently seemed insurmountable.

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